Cultivos Tropicales, Vol. 47, No. 1, January-March 2026, e-ISSN: 1819-4087, p-ISSN: 0258-5936, https://ediciones.inca.edu.cu

Cu-ID: https://cu-id.com/2050/v47n1e10

Original article

Green synthesis of silver nanoparticles (AgNPs) using *Rosmarinus officinalis* extract and evaluation of their preservative effect on *Malus domestica*

Síntesis verde de nanopartículas de plata (AgNPs) utilizando extracto de *Rosmarinus officinalis* y evaluación de su efecto conservante en *Malus domestica*

Viviana Lorena Sánchez Vásquez[1*], Yessenia Beatriz Sarango Ortega[1], José Humberto Vera Rodríguez[2]

[1]Universidad Estatal de Milagro. Cdla Universitaria Dr. Rómulo Minchala Murillo - Km 1.5 vía Milagro - Virgen de Fátima, Milagro, Guayas, Ecuador, 091050.

[2]Universidad Agraria del Ecuador UAE. Vía Puerto Marítimo - Avenida 25 de Julio y Pío Jaramillo (Campus principal) Guayaquil, Guayas, Ecuador, 091307.

ABSTRACT: Green synthesis of silver nanoparticles (AgNPs) using plant extracts represents a sustainable approach for food preservation applications. In this study, aqueous and ethanolic extracts of *Rosmarinus officinalis* were employed as reducing and stabilizing agents for the formation of AgNPs, which were characterized by UV-Vis spectroscopy, showing surface plasmon resonance peaks between 415 and 443 nm. Phytochemical screening confirmed the presence of key bioactive metabolites, polyphenols, flavonoids, tannins, and saponins that supported nanoparticle formation and stability. Antimicrobial activity, assessed through disk diffusion against *Escherichia coli* ATCC 25922, revealed larger inhibition zones in ethanolic formulations, indicating a synergistic effect between extract constituents and AgNPs. When applied to *Malus domestica* fruits, the hybrid extract AgNPs system effectively reduced browning, firmness loss, and microbial deterioration during 15 days of storage. These findings highlight the potential of naturally derived AgNPs as a functional and sustainable alternative for extending the shelf life of fresh fruits and enhancing postharvest preservation technologies.

Key words: green synthesis, postharvest preservation, antimicrobial activity, phytochemicals.

RESUMEN: La síntesis verde de nanopartículas de plata (AgNPs) utilizando extractos vegetales constituye una alternativa sostenible para aplicaciones en conservación de alimentos. En este estudio se empleó extracto acuoso y etanólico de *Rosmarinus officinalis* como agente reductor y estabilizante para la obtención de AgNPs, las cuales fueron caracterizadas mediante espectroscopía UV-Vis, obteniendo picos de resonancia plasmónica entre 415 y 443 nm. Los extractos revelaron la presencia de metabolitos fitoquímicos clave, polifenoles, flavonoides, taninos y saponinas que favorecieron la formación y estabilidad de las nanopartículas. La actividad antimicrobiana, evaluada por difusión en disco contra *Escherichia coli* ATCC 25922, mostró halos de inhibición superiores en las formulaciones basadas en extracto etanólico, evidenciando un efecto sinérgico entre los compuestos bioactivos y las AgNPs. En la aplicación pos cosecha sobre manzanas (*Malus domestica*), las nanopartículas contribuyeron a reducir el pardeamiento, la pérdida de firmeza y el deterioro microbiano durante 15 días de almacenamiento. Estos resultados confirman el potencial del sistema extracto AgNPs como alternativa natural y funcional para la preservación de frutas frescas, promoviendo tecnologías poscosecha más seguras y sostenibles.

Palabras clave: conservación poscosecha, actividad antimicrobiana, fitoquímicos.

*Author for correspondence: vsanchezv@unemi.edu.ec

Received: 09/07/2025

Accepted: 25/10/2025

Conflict of Interest. Authors declare that they have no conflict of interest.

Author´s Contributions: Conceptualization: Viviana Sánchez-Vásquez, José Humberto Vera-Rodríguez. **Investigation:** Viviana Sánchez-Vásquez, Yessenia Beatriz Sarango Ortega. **Supervision:** Viviana Sánchez-Vásquez, Yessenia Beatriz Sarango Ortega. **Draft Writing:** Viviana Sánchez-Vásquez, Yessenia Beatriz Sarango Ortega. **Writing, Final Editing, and Data Curation:** José Humberto Vera Rodríguez.

INTRODUCTION

The search for natural alternatives for the preservation of fresh foods has gained increasing relevance in recent years, driven by the demand for “clean label” products and by global concern regarding the rise of microorganisms resistant to conventional antimicrobial treatments (1). This issue has motivated interest in bioactive matrices of plant origin, recognized for their safety, availability, and compatibility with food systems. Among them, rosemary (*Rosmarinus officinalis*) stands out due to its high content of secondary metabolites such as polyphenols, flavonoids, and terpenoids, which are widely documented for their antioxidant and antimicrobial activities (2). These characteristics make this species a promising source for the development of functional agents aimed at the preservation of fruits and vegetables.

In parallel, advances in nanotechnology have enabled the generation of materials with antimicrobial properties significantly superior to those of their conventional counterparts. Silver nanoparticles (AgNPs) are particularly relevant due to their ability to interact with cell membranes, structural proteins, and essential intracellular components, causing alterations in critical metabolic processes of bacteria and fungi (3). However, traditional methods of nanoparticle synthesis, such as chemical reduction with sodium borohydride, citrate synthesis, or pyrolysis of metallic salts, often involve the use of toxic reagents, high temperatures, or the generation of environmentally harmful by-products, which limits their direct application in edible matrices (4).

In this context, green synthesis based on plant extracts constitutes a sustainable strategy that allows the production of nanoparticles through biological reduction and stabilization mechanisms, avoiding the use of hazardous substances and reducing the environmental impact of the process (4). The phytochemical compounds present in plant extracts not only facilitate nanoparticle formation but also provide additional bioactive properties, generating hybrid systems of interest for postharvest preservation. The combination of AgNPs with polyphenol-rich extracts can enhance the antimicrobial and antioxidant activity of the resulting material, favoring its application as a natural coating for highly perishable fruits.

Fresh apples (*Malus domestica*) exhibit marked susceptibility to physiological and microbial deterioration during storage, manifested through processes such as browning, loss of firmness, and colonization by environmental bacteria. These factors represent a challenge for the distribution and commercialization chain. The incorporation of bioactive agents in the form of films, coatings, or surface-applied suspensions has shown potential to extend their shelf life, especially when combining antioxidant and antimicrobial inhibition mechanisms (5).

Within this framework, the use of nanoparticles obtained through green synthesis emerges as a promising alternative for the control of pathogens such as *Escherichia coli*, a microorganism of importance in food safety. The integration of plant extracts with metallic nanoparticles opens new avenues to improve the stability, functionality, and antimicrobial performance of natural preservatives, reducing dependence on synthetic additives and incorporating principles aligned with sustainability. A comprehensive analysis of these hybrid systems makes it possible to identify their capacity to mitigate microbial and physiological deterioration in fresh products, contributing to the development of safer, more efficient, and environmentally responsible postharvest technologies.

MATERIALS AND METHODS

Plant, Fruit Materials, and Reagents

Fresh leaves of *Rosmarinus officinalis* were purchased at “La Dolorosa” market (Milagro, Ecuador), selecting only material with uniform green coloration, free of necrosis or mechanical damage. Plant material was transported in thermal containers at 4 °C and processed within two hours of acquisition. Apples (*Malus domestica* var. “Anna”) were obtained from Tía supermarket (Babahoyo, Ecuador) and selected for uniform size (145-165 g), firmness ≥ 70 N measured with a FruitTest FT-327 penetrometer, uniform maturity, and absence of coatings, verified by hot water solubility test (60 °C, 30 s). Silver nitrate ($AgNO_3$, 99.8 %, Loba Chemie®), sodium hypochlorite (Merck®), ethanol 96 % (Quifatex®), phytochemical analysis reagents (Sigma-Aldrich®), Mueller-Hinton Agar (Oxoid®), and nutrient agar BD Difco™ were used. Pyrex® glassware, Falcon® tubes, and Whatman No. 1 discs were sterilized in a Tomy SX-500E autoclave.

Preparation of *Rosmarinus officinalis* extract

Leaves were washed with potable water and disinfected with 0.1 % sodium hypochlorite for 10 min, followed by three rinses with sterile distilled water. Plant material was ground with an Oster® 600 W electric grinder to obtain particles of 1-3 mm. For the first decoction, 150 g of ground material were heated with 300 mL distilled water in a Memmert WNB14 water bath at 95 ± 2 °C for 25 min. The mixture was hot-filtered with sterile gauze. Subsequently, 150 g of additional ground leaves were added to the filtrate and the decoction process was repeated. The combined extract was concentrated on a Thermo Scientific™ Cimarec+ hot plate at 60 °C for 20-25 min until reaching a final volume of 150 mL, and stored in amber Schott® bottles at 4 °C for up to 48 h.

Green synthesis of silver nanoparticles

AgNPs were synthesized by mixing 50 mL of concentrated plant extract with 50 mL of 0.02 M aqueous $AgNO_3$ solution in a sterile Erlenmeyer flask. The reaction was maintained at 60 °C under magnetic stirring at 400 rpm for 45 min. Nanoparticle formation was evidenced by a color change from pale yellow to reddish-brown. The resulting suspension was filtered with Whatman No. 1 paper and stored at 4 °C until characterization.

Spectroscopic Characterization (UV-Vis)

Characterization was performed using a Shimadzu UV-1900i spectrophotometer. Two milliliters of AgNP suspension were placed in Hellma Analytics® quartz cuvettes, and a spectral scan from 200 to 800 nm was carried out with 1 nm step interval, using distilled water as blank. The presence of the Surface Plasmon Resonance (SPR) peak confirmed the formation of metallic nanoparticles.

Qualitative phytochemical analysis

Phytochemical analysis of aqueous, acidified, and ethanolic extracts was performed using conventional methods to detect secondary metabolites.

Saponins: Foam test, shaking 5 mL extract for 30 s and observing foam stability after 10 min.

Phenolic compounds: Mixing 1 mL extract with three drops of 1 % $FeCl_3$ and recording characteristic color changes.

Flavonoids: Shinoda reaction, adding a fragment of metallic magnesium and concentrated HCl to 2 mL extract.

Tannins: Adding 1 % gelatin and 10 % NaCl to 2 mL extract, considering precipitate formation as positive.

Reducing sugars: Fehling's test, heating the mixture at 90 °C for 5 min and observing brick-red precipitate formation.

All tests were performed in duplicate with distilled water as blank.

Antimicrobial activity evaluation

Antimicrobial activity was evaluated against *Escherichia coli* ATCC 25922 using the disk diffusion method. The strain was reactivated in BD Difco™ nutrient agar at 37 °C for 24 h and adjusted to 0.5 McFarland standard with a Grant Instruments® BioDenser 2 densitometer. Mueller-Hinton Agar plates were inoculated with 100 µL of inoculum by spread technique, and Whatman No. 1 discs impregnated with 20 µL of each AgNP formulation were placed. Negative control (extract without nanoparticles) and positive control (gentamicin 10 µg) were included. Plates were incubated at 37 °C for 24 h, and inhibition zones were measured with a Mitutoyo® digital caliper.

Statistical analysis

Data obtained for absorbance, wavelength, inhibition zones, and physicochemical parameters were processed using descriptive statistics, reporting mean ± standard deviation (n = 3). Analysis was performed with R software, version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). All graphs and associated calculations were generated within the same statistical environment.

Postharvest preservation trial in apples

The preservative effect was evaluated in a completely randomized design with three treatments: negative control (no coating), positive control (beeswax emulsion), and treatment with AgNP-rosemary suspension. The beeswax emulsion was prepared by melting 5 g of BeeFarm® beeswax at 90 °C and emulsifying with 250 mL of boiling distilled water, stirring at 800 rpm for 30 min. Apples were disinfected with chlorinated water (10 ppm), dried in a Telstar Bio-II-A laminar flow cabinet, and each fruit was treated with 1.5 mL of the corresponding formulation using a Preval® atomizer. After drying for 30 min at 25 ± 2 °C, fruits were stored for 15 days at room temperature. Weight loss, firmness (FT-327 penetrometer), surface browning, and visual appearance were evaluated, with photographic records obtained using a Canon EOS T7 camera under controlled lighting.

RESULTS

Visual and spectroscopic characterization of silver nanoparticles (AgNPs)

The green synthesis of AgNPs using aqueous extract of *Rosmarinus officinalis* showed immediate visual evidence of reduction, reflected in the transition from the pale yellow color of the extract to reddish-brown shades after the heating process. This chromatic change is consistent with the formation of metallic nanoparticles and was observed homogeneously across all prepared formulations.

Characterization by UV-Visible spectrophotometry presented a well-defined Surface Plasmon Resonance (SPR) peak between 415 and 443 nm, with variations depending on the extract-$AgNO_3$ ratio. The spectra exhibited symmetric curves without lateral shoulders, suggesting a relatively homogeneous particle size distribution. The maximum absorbance ranged from 2.257 ± 0.011 to 2.334 ± 0.009, indicating high colloidal stability.

Table 1. UV-Vis spectroscopy of AgNPs synthesized with different extract-$AgNO_3$ ratios

Extract-$AgNO_3$	Wavelength (nm)	Absorbance (au)
10:5	440 ± 1	2.263 ± 0.012
5:5	424 ± 2	2.309 ± 0.010
10:5 (AA)	443 ± 1	2.257 ± 0.011
5:5 (AA)	426 ± 1	2.302 ± 0.014
10:5 (AE)	415 ± 3	2.334 ± 0.009
5:5 (AE)	418 ± 2	2.328 ± 0.011

AD = distilled extract, AA = acidulated extract, AE = ethanolic extract

Qualitative phytochemical analysis

The preliminary phytochemical analysis revealed the presence of secondary metabolites relevant to the green synthesis of nanoparticles. Table 2 presents the results obtained through qualitative tests for three types of extracts (distilled, acidulated, and ethanolic).

These results confirm differences in the availability of reducing metabolites depending on the solvent used, which is consistent with the spectral variations observed in the UV-Vis characterization.

Table 2. Presence of secondary metabolites in *Rosmarinus officinalis* extracts

Metabolites	Distilled extract (AD)	Acidulated extract (AA)	Ethanolic extract (AE)
Saponins	+	-	+
Reducing sugars	+	+++	+
Phenolic compounds	+++	++	+++
Flavonoids	++	++	+++
Tannins	++	+	+++
Terpenes	+	+	++

(+ = low; ++ = moderate; +++ = high)

Antimicrobial activity of AgNPs against *Escherichia coli* ATCC 25922

The antimicrobial activity evaluated by the disk diffusion method revealed significant inhibition zones for all AgNP formulations derived from the three types of extracts. The ethanolic extract (AE) showed the highest values, reaching inhibition zones of 16.2 ± 0.5 mm, while the distilled (AD) and acidulated (AA) extracts exhibited moderate activities, ranging between 5.1 ± 0.3 mm and 11.8 ± 0.4 mm.

Table 3. Antimicrobial Activity of AgNP-Rosemary fomulations against *E. coli* ATCC 25922

Treatment	Inhibition Zone (mm)	Mean ± SD	Tukey ($p \leq 0.05$)
AE 10:5	16 mm	16.2 ± 0.5	a
AE 5:5	14 mm	14.0 ± 0.6	a
AD 10:5	9 mm	9.1 ± 0.4	b
AD 5:5	6 mm	5.8 ± 0.3	b
AA 10:5	11 mm	11.8 ± 0.4	b
AA 5:5	5 mm	5.2 ± 0.2	b

The statistical analysis showed significant differences among treatments (ANOVA, $p < 0.05$). The formulations derived from the ethanolic extract exhibited the highest antimicrobial activity.

Postharvest preservation of apples treated with AgNP-Rosemary

During the 15-day period at room temperature, the fruits in the negative control group showed progressive deterioration starting on day 5, characterized by surface browning, loss of turgor, and the appearance of spots. The positive control (beeswax) exhibited a moderate delay in deterioration. Apples treated with the AgNP-rosemary suspension maintained their organoleptic characteristics throughout the experimental period, with lower weight loss and greater firmness compared to the controls.

The AgNP-rosemary treatment showed significantly higher values in firmness ($p < 0.05$) and weight retention, in addition to substantially lower browning

Table 4. Physicochemical evaluation of apples during storage (day 15)

Treatment	Weight Loss (%)	Firmness (n)	Browning (0-4)
Negative control	12.8 ± 0.7	52.1 ± 1.2	3.5 ± 0.2
Positive control	8.4 ± 0.5	59.0 ± 1.0	2.6 ± 0.3
AgNPs-Rosemary	4.1 ± 0.4	67.8 ± 1.4	0.8 ± 0.1

DISCUSSION

The results demonstrate that *Rosmarinus officinalis* extracts possess remarkable potential for the green synthesis of silver nanoparticles, which is consistent with studies highlighting the usefulness of natural compounds as sustainable alternatives for food preservation and microbial control (6). The presence of secondary metabolites identified in the extracts indicates a relevant role in nanoparticle reduction and stabilization processes, aligning with reports that attribute to polyphenols and terpenoids a significant capacity to modulate redox reactions and promote the formation of stable nanostructured assemblies (7). The range of plasmon resonance peaks observed in this study (415-443 nm) is consistent with typical values described for silver nanoparticles synthesized using plant extracts rich in phenolic compounds (8). The variability recorded between aqueous and ethanolic extracts is related to differences in the phytochemical composition extracted, a phenomenon widely recognized in the literature, where solvent polarity determines extraction efficiency and the nature of predominant metabolites (9). Regarding antimicrobial activity, the inhibition zones obtained against *Escherichia coli* confirm a synergistic effect between extract metabolites and silver nanoparticles. This synergy has been previously documented, where the combination of polyphenolic agents with nanometals enhances membrane permeabilization, structural destabilization, and oxidative stress generation in pathogenic microorganisms (10). Moreover, the superiority observed in treatments based on ethanolic extracts is consistent with studies indicating that organic solvents favor the extraction of compounds with greater antioxidant and antimicrobial activity (3). The preservative effect observed in fresh apples treated with the hybrid AgNP-extract formulation represents a relevant outcome for postharvest applications. The reduction in browning, firmness loss, and microbial damage aligns with research highlighting the usefulness of bioactive coatings and metallic nanoparticles to prolong fruit quality during storage (5). This evidence is consistent with recent developments aimed at edible films and functionalized coatings capable of incorporating natural antimicrobial and antioxidant agents (10). Finally, nanotechnology applied to food systems continues to position itself as a strategy with significant potential to improve the safety and shelf life of perishable products. However, aspects related to stability, migration, and consumer perception must be considered for future industrial implementation. Recent studies emphasize the importance of evaluating these factors as part of the responsible development of nanoparticle-based technologies for food use (9).

CONCLUSIONS

Rosmarinus officinalis extract constitutes an effective bioactive agent for the green synthesis of silver nanoparticles, due to its richness in polyphenols, flavonoids, and other secondary metabolites capable of reducing and stabilizing metal ions without the need for aggressive chemical reagents. The AgNPs generated exhibited optical characteristics consistent with well-formed and stable nanoparticles, with plasmon resonance peaks within the characteristic range for silver synthesized using plant species.

The antimicrobial evaluation revealed that the hybrid extract-AgNP formulations exerted significant inhibitory activity against *Escherichia coli*, confirming a synergistic effect between the phytochemical compounds of the extract and the biocidal action of silver. This behavior was reinforced in treatments obtained with ethanolic extract, which showed larger inhibition zones and greater consistency in microbial response.

In postharvest application, the incorporation of these nanoparticles into surface treatments of fresh apples reduced browning, decreased firmness loss, and limited visible microbial colonization during storage, thereby extending fruit stability and commercial appearance. These findings support the potential of plant-derived AgNPs as a natural and sustainable alternative for the preservation of perishable fruits. Overall, the study confirms the technical feasibility of integrating rosemary extracts and silver nanoparticles obtained through green synthesis into postharvest preservation strategies, offering a functional option with antimicrobial and antioxidant properties that could partially or fully replace synthetic preservatives. To advance toward industrial implementation, further studies are recommended on migration analysis, long-term stability, toxicological evaluation, and consumer acceptance, in order to ensure the safety and sustainability of their use in real food systems.

BIBLIOGRAPHY

1. Novais, C.; Molina, A.K.; Abreu, R.M.V.; Santo-Buelga, C.; Ferreira, I.C.F.R.; Pereira, C.; Barros, L. Natural I. J. Agric. Food Chem. 2022, 70, 2789-2805. Available from: doi: http://doi.org/10.1021/acs.jafc.1c07533.
2. Meziane, H.; Zraibi, L.; Albusayr, R.; Bitari, A.; Oussaid, A.; Hammouti, B.; Touzani, R. *Rosmarinus Officinalis* Linn. Unveiling its multifaceted nature in nutrition, diverse applications, and advanced extraction methods. *J. U*mm Al-Qura Univ. Appl. Sci. 2025, 11, 9-37. Available from: doi: http://doi.org/10.1007/s43994-024-00144-y.
3. Adeyemi, J.O.; Fawole, O.A. Metal-based nanoparticles in food packaging and coating technologies: A Review. Biomolecules 2023, 13, 1092. Available from: doi: http://doi.org/10.3390/biom13071092.
4. Pardo, L.; Arias, J.; Molleda, P. Elaboración de nanopartículas de plata sintetizadas a partir de extracto de hojas de romero (*Rosmarinus Officinalis* L.) y su uso como conservante. La Granja 2021, *35*. Available from: doi: http://doi.org/10.17163/lgr.n35.2022.04.
5. Solano-Doblado, L.G.; Alamilla-Beltrán, L.; Jiménez-Martínez, C. Películas y recubrimientos comestibles funcionalizados. TIP Rev. Espec. In Cienc. Quím.-Biológicas. 2018, 21, 30-42. Available from: doi: http://doi.org/10.22201/fesz.23958723e.2018.0.153.
6. Karnwal, A.; Malik, T. Exploring the untapped potential of naturally occurring antimicrobial compounds: novel advancements in food preservation for enhanced safety and sustainability. Front. Sustain. *Food Syst.* 2024, *8*, 1307210. Available from: doi: http://doi.org/10.3389/fsufs.2024.1307210.
7. Liao, Z.; Ali, M.H.; Tu, D.; Xiong, S.; Suleiman, N. Public Acceptance on nanotechnology in edible food material: An Empirical Study from China. Int. J. Econ. Manag. 2025, 19, 67-84. Available from: doi: http://doi.org/10.47836/ijeam.19.1.05.
8. Flores-Villa, E.; Sáenz-Galindo, A.; Castañeda-Facio, A.O.; Narro-Céspedes, R.I. Romero (*Rosmarinus Officinalis* L.): Su origen, importancia y generalidades de sus metabolitos secundarios. TIP Rev. Espec. En Cienc. Quím.-Biológicas 2020, *23.* Available from: doi: http://doi.org/10.22201/fesz.23958723e.2020.0.266.
9. Guidotti-Takeuchi, M.; De Morais Ribeiro, L.N.D.M.; Dos Santos, F.A.L.; Rossi, D.A.; Lucia, F.D.; De Melo, R.T. Essential oil-based nanoparticles as antimicrobial agents in the food industry. Microorganisms 2022, 10, 1504. Available from: doi: http://doi.org/10.3390/microorganisms10081504.
10. Colín-Álvarez, M.D.L.; Calderón-Domínguez, G.; Rojas-Candelas, L.E.; Rentería-Ortega, M. Revisión: Aplicación de la nanotecnología como innovación en recubrimientos alimentarios. Pädi Bol. Científico Cienc. Básicas E Ing. ICBI 2024, 12, 21-33. Available from: doi: http://doi.org/10.29057/icbi.v12iEspecial.12127.